PATENT
Docket No. 300622004620

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Nora Durant

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Rajesh GOKHALE et al.

Serial No.: 10/091,244

Filing Date: 04 March 2002

For: METHODS TO MEDIATE POLYKETIDE SYNTHASE MODULE EFFECTIVENESS

Examiner: To be assigned

Group Art Unit: 1652

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

BOX MISSING PARTS
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is in response to the Notice to File Missing Parts of Nonprovisional Application mailed April 25, 2002, for which a response was due on June 25, 2002. Accordingly, a one month petition for extension of time is filed herewith, extending the time for response to July 25, 2002.

Please enter the following sequence listing, amendments and remarks.

07/30/2002 HBERHE 00000070 031952 10091244
02 FC:215 55.00 CH

sd-96685

In the Sequence Listing:

Please insert the attached paper copy of the Sequence Listing as new pages 1-7 in the above-captioned application. A computer-readable form (CRF) copy of the Sequence Listing accompanies this response.

AMENDMENT

In the Specification:

Please replace paragraph [00113] with the following rewritten paragraph [00113]:

--The N-terminal linker of M3 was synthesized by New England Peptide (Fitchburg, MA). The peptide sequence was as follows, M3 N-term: H_2N-MTDSEKVAEYLRRATLDLRAARQRIRELESD-amide. (SEQ ID NO: 20)--

Please replace paragraph [00114] with the following rewritten paragraph [00114]:

--**Construction of Plasmids.** Plasmid pBP19 contains module 2 of DEBS (M2) and is a derivative of pRSG64 (Gokhale, R. S., *et al.*, (1999) *Science 284*, 482-485), where the thioesterase domain was replaced with a *Spe*I-*Eco*RI fragment containing the natural C-terminal linker for module 2 to make pBP19. Plasmid pST179 encodes a derivative of M2 containing the C-terminal linker of DEBS module 4 (M4). The C-terminal linker of M4 was obtained as a *Spe*I-*Eco*RI fragment by PCR using the primers 5'-ACT AGT **AGG CTG TTC GCG GCC TCA C**-3' (SEQ ID NO: 21) and 5'-G GGA ATT **CAG GTC CTC TCC CCC GC**-3' (SEQ ID NO: 22) (bold sequences complement DEBS sequence). The PCR amplicon was inserted after M2 using the engineered sites, yielding pST179. This plasmid, pRSG34, encodes module 3 of DEBS (M3) with its own N-terminal linker and with the thioesterase fused to the C-terminus. Its construction has been described previously (*id.*). Plasmid pST132 encodes a derivative of M3 + TE, where the natural N-terminal linker of pRSG34 has been replaced with the N-terminal linker of module

5 of DEBS (M5). This substitution required the replacement of the *Nde*I-*Bsa*BI fragment of pRSG34 with the corresponding fragment from pJRJ10 (Jacobsen, J. R., *et al.*, (1998) *Biochemistry 37*, 4928-4934). All constructs were cloned into pET-21c (Novagen) vectors for expression in *Escherichia coli*.--

Please replace paragraph [00131] with the following rewritten paragraph [00131]:

--**Construction of Plasmids.** The gene encoding ACP4(4) was amplified as an *Nde*I-*Eco*RI PCR fragment (523 bp) using the primers 5'-CCATATG**GTGGTCGACCGGCTCG**-3' (SEQ ID NO: 23) and 5'-GAATTC**CTA-CAGGTCCTCTCCCCC**- 3'(SEQ ID NO: 24) (sequences complementary to DEBS shown in bold). The PCR product was cloned into pET28a (Novagen) to yield plasmid pNW8. Plasmid pST157 encodes a bimodular fusion between module 1 of DEBS1 and module 5 of DEBS3, with the thioesterase domain fused downstream of module 5 ("M1+M5+TE"). This fusion, which was engineered by taking advantage of the natural, conserved *Bsa*BI sites located at the start of the KS domains of modules 2 and 5, also includes the loading didomain of DEBS1. The "linker" sequence that covalently bridges the fused modules is the natural sequence between modules 1 and 2, as in DEBS1. The fusion junction between module 5 and the thioesterase domain is identical to that in plasmid pRSG46.[23] Similarly, plasmid pST92 encodes an "M1+M6+TE" bimodular fusion. Its construction, which is completely analogous to that of pST157, involves introduction of this bimodular PKS gene from pST96, Gokhale, et al., *Science* 1999, *284*, 482-485, as an *Nde*I-*Eco*RI into pET-21c (Novagen). The construction of genes encoding (5)M2+TE, (3)M3+TE, (5)M5+TE, and (5)M6+TE (pRSG64, pRSG64, pRSG46, and pRSG54, respectively) have been described previously, *id.*, as well as the construction of a gene encoding (5)M3+TE (pST132). *See* Tsuji, et al., *Biochemistry* 2001.--

REMARKS

The Specification has been amended to include sequence identification numbers which were omitted at the time of filing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**".

The undersigned hereby states that the paper copy of the Sequence Listing and the computer readable form copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.825(a) and (b), respectively, are the same and contain no new matter. Accordingly, entry of the Sequence Listing into the above-captioned case is respectfully requested.

In the unlikely event that the patent office determines that extensions and/or other relief is required, applicant petition for any required relief including extensions of time and authorize the assistant commissioner to charge the cost of such petitions and/or fees due to our deposit account no. 03-1952 under order no. 300622004620. The assistant commissioner is not authorized to charge the cost of the issue fee to the deposit account.

Respectfully submitted,

Dated: July 24, 2002

By: Brenda J. Wallach

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph [00113] at page 31 has been amended as follows:

The N-terminal linker of M3 was synthesized by New England Peptide (Fitchburg, MA). The peptide sequence was as follows, M3 N-term: H_2N-MTDSEKVAEYLRRATLDLRAARQRIRELESD-amide. (SEQ ID NO: 20)

Paragraph [00114] at page 32 has been amended as follows:

Construction of Plasmids. Plasmid pBP19 contains module 2 of DEBS (M2) and is a derivative of pRSG64 (Gokhale, R. S., *et al.*, (1999) *Science 284*, 482-485), where the thioesterase domain was replaced with a *Spe*I-*Eco*RI fragment containing the natural C-terminal linker for module 2 to make pBP19. Plasmid pST179 encodes a derivative of M2 containing the C-terminal linker of DEBS module 4 (M4). The C-terminal linker of M4 was obtained as a *Spe*I-*Eco*RI fragment by PCR using the primers 5'-ACT AGT **AGG CTG TTC GCG GCC TCA C**-3' (SEQ ID NO: 21) and 5'-G GGA ATT **CAG GTC CTC TCC CCC GC**-3' (SEQ ID NO: 22) (bold sequences complement DEBS sequence). The PCR amplicon was inserted after M2 using the engineered sites, yielding pST179. This plasmid, pRSG34, encodes module 3 of DEBS (M3) with its own N-terminal linker and with the thioesterase fused to the C-terminus. Its construction has been described previously (*id.*). Plasmid pST132 encodes a derivative of M3 + TE, where the natural N-terminal linker of pRSG34 has been replaced with the N-terminal linker of module 5 of DEBS (M5). This substitution required the replacement of the *Nde*I-*Bsa*BI fragment of pRSG34 with the corresponding fragment from pJRJ10 (Jacobsen, J. R., *et al.*, (1998) *Biochemistry 37*, 4928-4934). All constructs were cloned into pET-21c (Novagen) vectors for expression in *Escherichia coli*.

Paragraph [00131] at page 40 has been amended as follows:

Construction of Plasmids. The gene encoding ACP4(4) was amplified as an *NdeI-EcoRI* PCR fragment (523 bp) using the primers 5'-CCATATG**GTGGTCGACCGGCTCG**-3' (SEQ ID NO: 23) and 5'-GAATTC**CTA-CAGGTCCTCTCCCCC**- 3'(SEQ ID NO: 24) (sequences complementary to DEBS shown in bold). The PCR product was cloned into pET28a (Novagen) to yield plasmid pNW8. Plasmid pST157 encodes a bimodular fusion between module 1 of DEBS1 and module 5 of DEBS3, with the thioesterase domain fused downstream of module 5 ("M1+M5+TE"). This fusion, which was engineered by taking advantage of the natural, conserved *Bsa*BI sites located at the start of the KS domains of modules 2 and 5, also includes the loading didomain of DEBS1. The "linker" sequence that covalently bridges the fused modules is the natural sequence between modules 1 and 2, as in DEBS1. The fusion junction between module 5 and the thioesterase domain is identical to that in plasmid pRSG46.[23] Similarly, plasmid pST92 encodes an "M1+M6+TE" bimodular fusion. Its construction, which is completely analogous to that of pST157, involves introduction of this bimodular PKS gene from pST96, Gokhale, et al., *Science* 1999, *284*, 482-485, as an *Nde*I-*Eco*RI into pET-21c (Novagen). The construction of genes encoding (5)M2+TE, (3)M3+TE, (5)M5+TE, and (5)M6+TE (pRSG64, pRSG64, pRSG46, and pRSG54, respectively) have been described previously, *id.*, as well as the construction of a gene encoding (5)M3+TE (pST132). *See* Tsuji, et al., *Biochemistry* 2001.